FEBS Letters 584 (2010) 1628–1632

journal homepage: www.FEBSLetters.org

KatG from *Salmonella* Typhimurium is a peroxynitritase

Samantha McLean *, Lesley A.H. Bowman, Robert K. Poole

Department of Molecular Biology and Biotechnology, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK

ARTICLE INFO

Article history:
Received 8 March 2010
Revised 16 March 2010
Accepted 16 March 2010
Available online 19 March 2010

Edited by Stuart Ferguson

Keywords:
Peroxynitrite
Dihydrorhodamine
Catalase
Peroxidase
Peroxynitritase

ABSTRACT

Pathogenic bacteria elicit protective responses to oxidative and nitrosative stresses. Although such responses are generally distinct, it was recently reported in *Mycobacterium tuberculosis* that catalase–peroxidase (KatG), a classical defence against peroxides, also exhibits peroxynitritase activity. Here, the *katG* gene from *Salmonella* Typhimurium was cloned and protein purified and characterised. An increase in the rate of decomposition of peroxynitrite was observed for KatG from the enterobacterium with a second-order rate constant of $4.2 \times 10^4\ M^{-1}\ s^{-1}$ at pH 7.4, 25 °C. This enzyme was able to reduce dihydrorhodamine oxidation by peroxynitrite to ~83%. Given the peroxynitritase activity demonstrated here it is likely that KatG may play a wider role in the detoxification of oxidative stresses than previously thought.

1. Introduction

Enteropathogens are capable of causing acute intestinal inflammation that manifests with diarrhoea and vomiting. In response to infection, the host innate immune system, primarily macrophages, produces reactive oxygen and nitrogen species, including nitric oxide (·NO), superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) as well as the subsequent production of secondary species including peroxynitrite due to their inherent reactivity (reviewed in [1–3]). Peroxynitrite is formed by the near diffusion-limited reaction of $O_2^{\cdot-}$ with ·NO. Previous studies have shown that this highly reactive species, which has a half-life of only 1 s at pH 7.4, is able to react with invading pathogens both inside and within the vicinity of the macrophage and would thus be encountered by these pathogens [4]. Enterobacteria have developed mechanisms of resistance to reactive oxygen and nitrogen species in order to survive and proliferate within the host. One such mechanism is the increase in expression of a catalase–peroxidase (KatG), which is able to alleviate discrete forms of oxidative stress via the breakdown of H_2O_2 and other hydroperoxides into harmless secondary species [5,6].

The mechanisms of both $O_2^{\cdot-}$ and ·NO detoxification have been studied in detail in enterobacterial systems (reviewed in [2,7]). However, relatively little is known about the breakdown of peroxynitrite by these organisms. A previous study into the activity of KatG from *Mycobacterium tuberculosis* has demonstrated that this enzyme is able to act as a peroxynitritase, increasing the rate of decomposition of peroxynitrite in aqueous solutions [8]. This work aimed to ascertain whether KatG isolated from *Salmonella enterica* serovar Typhimurium is also able to play a role in peroxynitrite detoxification.

2. Materials and methods

2.1. Materials

Unless otherwise stated, chemicals were obtained from Sigma–Aldrich Company Ltd. and were of analytical grade.

2.2. Construction of the pTrcHis-KatG plasmids

The oligonucleotides S-KatG-F: GTT CTT **GCT AGC** ATG AGC ACG ACC GAC GAT ACC CAT AAC and S-KatG-R: GTT CTT AAG CTT TTA TTG CAG ATC GAA ACG GTC CAG GTT were used to PCR amplify the *katG* gene from *Salmonella* Typhimurium ATCC 14028s genomic DNA and incorporate *Nhe*I (bold) and *Hin*dIII (underlined) restriction sites at either end of the gene. This gene was subsequently ligated into pre-digested pTrcHis A vector (Invitrogen™) by standard techniques.

Abbreviations: ·NO, nitric oxide; $O_2^{\cdot-}$, superoxide; H_2O_2, hydrogen peroxide; KatG, catalase–peroxidase; DTPA, diethylenetriaminepentaacetic acid; DHR, dihydrorhodamine 123

* Correspondence to: S. McLean, Department of Molecular Biology and Biotechnology, The University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK. Fax: +44 114 272 8697.
E-mail address: S.McLean@sheffield.ac.uk (S. McLean).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.03.029

2.3. Protein expression and purification

The plasmids were used to transform chemically competent JM109 *Escherichia coli* cells, which were subsequently grown in 1 L of Circlegrow® media supplemented with ampicillin (150 μg/mL) and hemin (20 mg/L) in 2 L baffled flasks for 20 h at 30 °C in a shaking incubator. Cells were harvested by centrifugation and resuspended in binding buffer (50 mM Tris–MOPS, pH 8.0 and 300 mM NaCl), plus a protease inhibitor cocktail (Sigma®, 1 mL/20 g wet cell weight).

Cells were lysed by sonication on ice and cell debris removed by centrifugation at 20 000×*g* (4 °C) for 30 min. The supernatant was loaded onto a pre-equilibrated Talon metal affinity column (Clontech) and washed with binding and wash (10 mM imidazole) buffers. The protein was eluted with 200 mM imidazole and subsequently desalted on a PD-10 desalting column with 50 mM Tris–MOPS, pH 8.0. KatG concentrations were determined using the molar extinction coefficient ε_{280} = 143 350 M^{-1} cm^{-1}.

2.4. Catalase, peroxidase and peroxynitritase assays

Catalase activity was monitored as previously described [9]; briefly, various concentrations of H_2O_2 were added to 5 nM KatG in 50 mM phosphate buffer pH 7.4 at 37 °C and catalase breakdown was monitored by a change in absorbance at 240 nm in a Cary 50 spectrophotometer over 30 s. The molar extinction coefficient of ε_{240} = 43.6 M^{-1} cm^{-1}, was used to calculate the concentration of H_2O_2 [10]. Peroxidase assays were performed as previously described [11]. Briefly, activity was monitored by measuring the rate of *o*-dianisidine oxidation in the presence of 23 mM *t*-butyl hydroperoxide by monitoring absorbance change at 460 nm (ε_{460} = 11 300 M^{-1} cm^{-1}).

Resistance of catalase and peroxidase activities to peroxynitrite was assayed by pre-incubating 1.5 μM enzyme with 0–375 μM peroxynitrite tetramethylammonium for 5 min. Samples were passed over a Sephadex G-25 column, diluted and the activity was measured as described above.

Peroxynitrite preparations were bought commercially from Calbiochem® and peroxynitrite tetramethylammonium was purchased from Alexis® biochemicals; concentrations were determined in 0.1 M NaOH, the molar extinction coefficient of ε_{302} = 1670 M^{-1} cm^{-1} was used to calculate substrate concentration. Peroxynitrite decomposition was monitored using an Applied Photophysics stopped flow spectrophotometer. Peroxynitrite solutions in 0.01 M NaOH were mixed in a 1:1 ratio with KatG buffered in 100 mM phosphate, pH 7.4 and 100 μM diethylenetriaminepentaacetic acid (DTPA). The breakdown of peroxynitrite was monitored by a decrease in absorbance at 302 nm at 25 °C.

2.4.1. Measurement of peroxynitrite-induced oxidation of dihydrorhodamine 123 (DHR)

Reactions were carried out in 100 mM phosphate buffer, pH 7.4 and 100 μM DTPA. The oxidation of 100 μM DHR to rhodamine was monitored by an absorbance change at 500 nm in the presence of various concentrations of KatG or catalase enzyme after a 1 min incubation. The reaction was initiated by the addition of 20 μM peroxynitrite.

2.5. Analysis of kinetic data

Steady-state rates of catalase and peroxidase activity were estimated by fitting time course data to a straight line. The steady-state rate data (rate versus substrate concentration) were subsequently fitted to single rectangular hyperbolae (Sigmaplot 10; SPSS) in order to determine k_{cat} and apparent K_m. Error bars represent SEM of the triplicate dataset and errors given for the kinetic parameters represent the standard errors produced when fitting the data. Apparent second-order rate constants of the reaction of KatG with peroxynitrite were determined from the slope of plotting KatG concentration versus rate of exponential decay at several fixed concentrations of KatG. Exponential decay curves represent the mean of >10 replicates.

3. Results

3.1. Expression and purification of recombinant KatG

The KatG enzyme was expressed and purified by affinity chromatography. The enzyme from *S.* Typhimurium was shown to be highly pure by SDS–PAGE (Fig. 1A). The absorbance spectra are shown in Fig. 1B. The peak at 280 nm represents protein while the peaks at 408, 507 and 631 nm represent bound haem in the high spin Fe^{3+} state [12].

3.2. Catalase and peroxidase activity

KatG catalyses the breakdown of H_2O_2 to water and oxygen. This was measured continuously by monitoring the absorbance of H_2O_2 at 240 nm. Various concentrations of substrate were assayed and steady-state rates were calculated using the linear

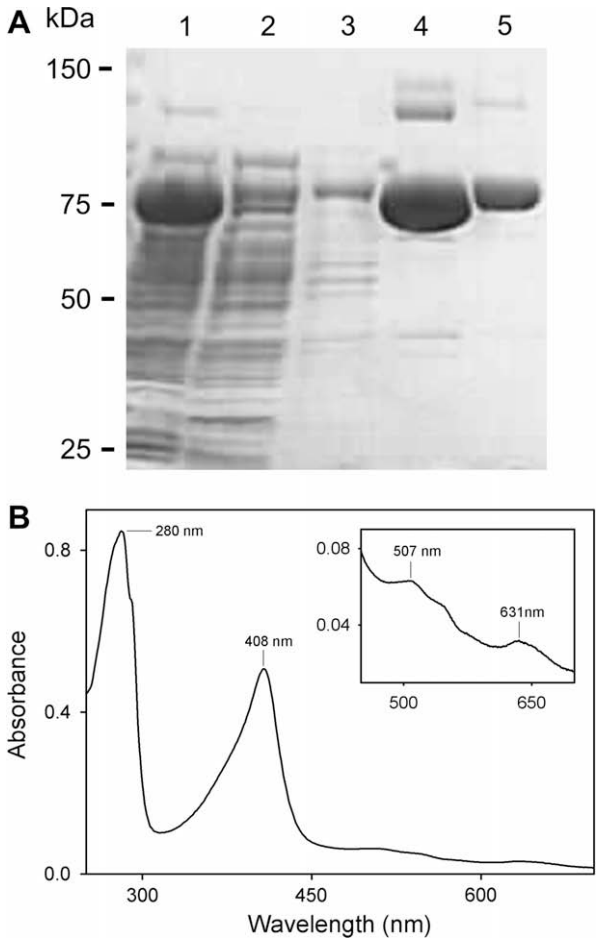

Fig. 1. Purification of KatG from *S.* Typhimurium. (A) SDS–PAGE showing the purification of KatG from *S.* Typhimurium prepared from JM109 competent cells containing the pTrcHisA-S-*katG* vector. Lane 1 – cell lysate; lane 2 – Talon column flow through; lane 3 – imidazole wash; lane 4 – pure protein eluted with 200 mM imidazole; lane 5 – pure protein after desalting. (B) Absolute absorbance spectra of 5.6 μM purified His_6-KatG protein from *S.* Typhimurium at pH 7.4.

gradient in a plot of time versus H_2O_2 concentration. These rates were plotted against substrate concentration to yield a simple hyperbolic curve (Fig. 2A), from which kinetic parameters could be estimated. The k_{cat} for H_2O_2 degradation and the apparent K_m for H_2O_2 were found to be $7.0 \pm 0.2 \times 10^3\ s^{-1}$ and 1.6 ± 0.2 mM, respectively. The k_{cat}/K_m was calculated as $4.4 \times 10^6\ M^{-1}\ s^{-1}$.

The peroxidatic activity of KatG was assayed as previously described [11], by monitoring the oxidation of *o*-dianisidine in the presence of *t*-butyl hydroperoxide. By varying the concentration of *o*-dianisidine and plotting substrate concentration versus reaction rate (Fig. 2B), the k_{cat} for *o*-dianisidine oxidation and apparent K_m for *o*-dianisidine were found to be $12.1 \pm 1.0\ s^{-1}$ and 0.20 ± 0.05 mM, respectively. The k_{cat}/K_m was calculated as $6.0 \times 10^4\ M^{-1}\ s^{-1}$.

Catalase and peroxidase activities were also assayed for their ability to resist peroxynitrite exposure. Both are relatively resistant to this species; when peroxynitrite was 250-fold in excess of 1.5 μM KatG, catalase activity dropped to ~89% and peroxidase activity to ~75% (Fig. 2C). There was a small rise in catalase activity upon addition of low levels of peroxynitrite.

3.3. Peroxynitritase activity

It has been previously reported that KatG from *M. tuberculosis* exhibits peroxynitritase activity [8]. Recent studies have also shown that bovine heart and liver catalases are able to enhance the rate of peroxynitrite decomposition [13,14]. The KatG enzyme was assayed for its ability to increase the rate of peroxynitrite breakdown by monitoring substrate decomposition via a decrease in absorbance at 302 nm in a stopped flow spectrophotometer. In the presence of KatG, the breakdown of peroxynitrite was enhanced (Fig. 3A); however, different preparations of peroxynitrite contain different contaminants and had to be controlled for. Two common contaminants of peroxynitrite preparations are nitrite and H_2O_2, which are unused substrates from synthesis [15]. To control for this, we measured the rate of peroxynitrite decay in the presence of KatG using peroxynitrite obtained from Calbiochem®, which had a nitrite concentration determined by the Greiss reaction as 12-fold higher than that of peroxynitrite, or peroxynitrite tetramethylammonium, which is synthesised from the reaction of nitrogen monoxide and tetramethylammonium superoxide [16] and contains ~1% nitrite and no H_2O_2. There was no significant difference between the rates of decay, measured in triplicate, using peroxynitrite from either source as determined by the Students *t*-test. There was also no significant difference when nitrite was added to the peroxynitrite tetramethylammonium assay in 100-fold excess. This suggests that nitrite and H_2O_2 do not play a role in the KatG enhanced breakdown of peroxynitrite.

Additionally, contaminants in the KatG preparations could influence the reaction. To determine whether small molecules were affecting the rate of peroxynitrite breakdown, reaction rates were determined in triplicate using both diluted and concentrated (Vivaspin 20 concentrator, molecular weight cut off of 30 kDa) KatG preparations. There was no significant difference in the rates as determined by the Students *t*-test suggesting that there was no effect on the rate of peroxynitrite decay by small molecules present in the KatG preparations. The rate was also unaltered when KatG samples were bubbled with nitrogen prior to assaying to remove any traces of CO_2 in the buffer. DTPA (100 μM) was added to assays to remove the possibility of free transition metals being responsible for peroxynitrite decay.

The rate of peroxynitrite decay was measured at several fixed concentrations of KatG; all traces were fitted to single exponential decays from which the rate constants (k_{obs}) could be determined. k_{obs} was linearly dependent on enzyme concentration and the sec-

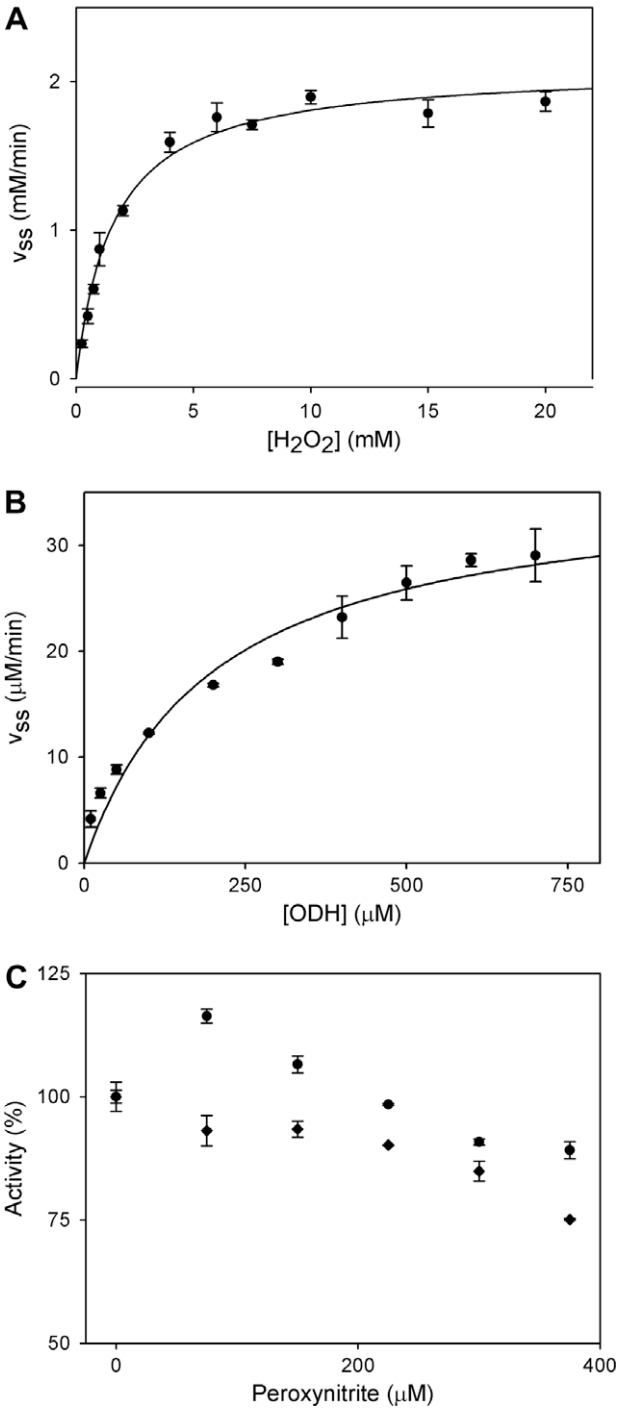

Fig. 2. Kinetic characterisation of *S.* Typhimurium KatG. (A) The catalase activity of *S.* Typhimurium KatG. Various concentrations of H_2O_2 were incubated in 50 mM phosphate buffer, pH 7.4 at 37 °C. The reaction was initiated by addition of 5 nM KatG and the reaction monitored by change in absorbance at 240 nm. Each rate (v_{ss}) was plotted against H_2O_2 concentration to give a simple hyperbolic curve from which kinetic parameters could be derived. Each rate was measured in triplicate and plots of the means ± S.E.M. are shown. (B) The peroxidase activity of *S.* Typhimurium KatG. 23 mM *t*-butyl hydroperoxide and 50 nM KatG were buffered in 50 mM phosphate buffer, pH 7.4 at 37 °C. The reaction was initiated by the addition of *o*-dianisidine and the absorbance monitored at 460 nm for 30 s. Initial rates (v_{ss}) were plotted against *o*-dianisidine concentration. Each rate was measured in triplicate and plots of the means ± S.E.M. are shown. (C) Catalase (●) and peroxidase (♦) activities were determined after 5 min pre-incubation with 0–375 μM peroxynitrite tetramethylammonium and are expressed as a percentage of the activity measured in the absence of peroxynitrite. Plots represent the means of three replicates ± S.E.M.

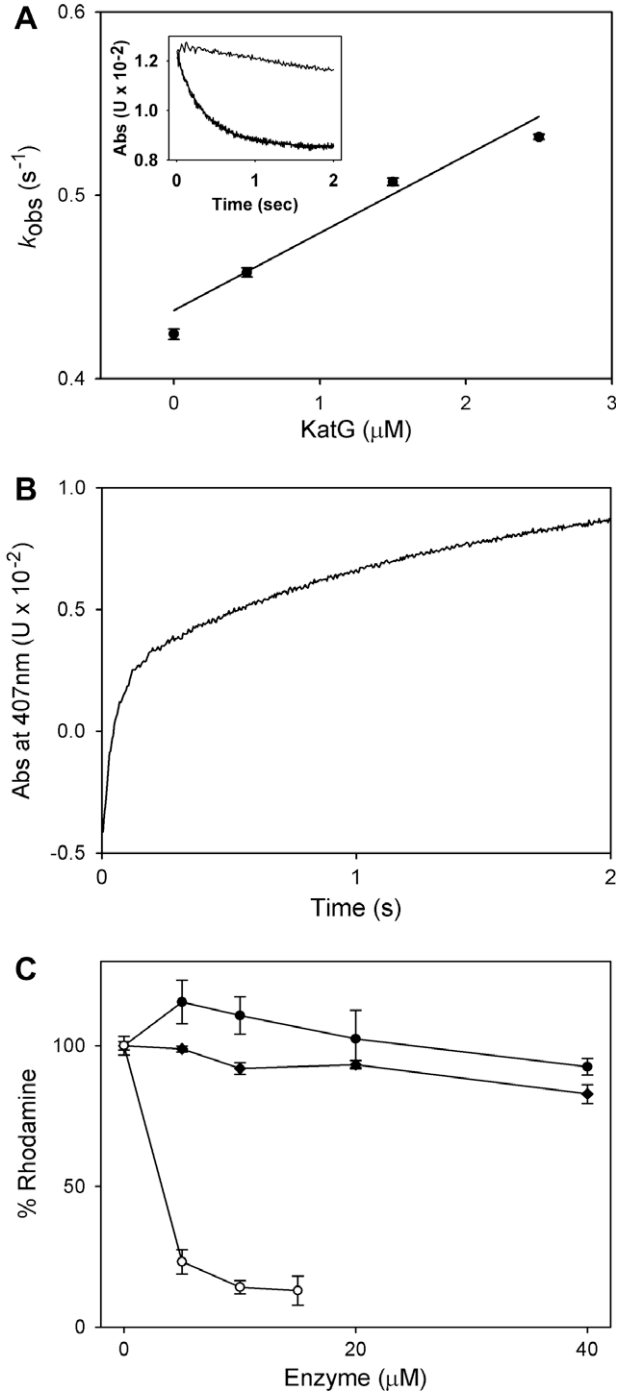

Fig. 3. KatG exposure to peroxynitrite. (A) Stopped flow measurements of peroxynitrite breakdown at 25 °C. Peroxynitrite (110 μM) in 0.01 M NaOH (final concentration) was mixed with 100 mM phosphate buffer (pH 7.4) and 100 μM DTPA containing varying concentrations of KatG from *S.* Typhimurium. All observed rates are the average of >10 assays ± S.E.; the apparent second-order rate constant was determined from the slope of these plots. Inset shows a time course of 10 μM peroxynitrite decomposition in the presence and absence of 10 μM KatG, monitored at 302 nm. (B) Time course of the reaction between KatG and peroxynitrite measured at 407 nm. KatG (10 μM) in 100 mM phosphate buffer (pH 7.4) and 100 μM DTPA was mixed with 10 μM peroxynitrite in 0.01 M NaOH at 25 °C; the reaction was recorded on a stopped flow spectrophotometer over 2 s. (C) The protective effect of KatG on DHR. The oxidation of 100 μM DHR was determined by absorbance at 500 nm after a 1 min incubation with 20 μM peroxynitrite and varying concentrations of KatG using peroxynitrite (●), peroxynitrite tetramethylammonium (♦) or bovine liver catalase and peroxynitrite (○) in 100 mM phosphate buffer (pH 7.4) and 100 μM DTPA. Assays were carried out at 25 °C.

Table 1
Second-order rate constants for the enzymatic catabolism of peroxynitrite. Second-order rate constants showing the relative activity of various enzymes known to act as peroxynitritases to catalyse the breakdown of peroxynitrite (NS – not stated).

Protein	Organism/ source	Temperature (°C)/pH	Second-order rate constant (M^{-1} s^{-1})
Peroxiredoxin V [26]	*H. sapien*	RT/7.8	7.0×10^7
Glutathione peroxidase [28]	Bovine erythrocytes	25/7.4	8.0×10^6
Myeloperoxidase [22]	*H. sapien*	12/7.2	6.2×10^6
Horseradish peroxidase [22]	*A. rusticana*	25/7.4	3.2×10^6
Catalase [13]	Bovine heart	25/7.4	1.6×10^6
AhpC [20]	*S.* Typhimurium	NS/6.8	1.5×10^6
Thioredoxin peroxidase I [27]	*S. cerevisiae*	25/7.4	7.4×10^5
Tryparedoxin peroxidase [25]	*T. cruzi*	37/7.4	7.2×10^5
Lactoperoxidse [22]	*H. sapiens*	12/7.4	3.3×10^5
KatG [8]	*M. tuberculosis*	37/7.4	1.4×10^5
KatG [this work]	*S.* Typhimurium	25/7.4	4.2×10^4

ond-order rate constant was calculated as 4.2×10^4 M^{-1} s^{-1} (Fig. 3A and Table 1) at pH 7.4 and 25 °C. This is approximately 3-fold lower than that of KatG from *M. tuberculosis* (1.4×10^5 M^{-1} s^{-1}, [8]). At all times substrate concentrations were kept in over 40-fold excess to confirm that the reaction between KatG and peroxynitrite was catalytic.

The reaction between KatG and peroxynitrite was followed at several fixed wavelengths (between 407 and 430 nm) using a stopped flow spectrophotometer in an attempt to identify intermediate species. Fig. 3B shows an absorbance decrease at the Soret band; this decrease occured within the 2 ms dead time of the stopped flow apparatus, so that the trace shows the subsequent increase in absorbance as KatG transitions back to native enzyme. A red shift was also observed, with a peak at 430 nm; no isosbestic wavelength between the native and red shifted peaks was found.

3.4. Protective effect of KatG on DHR

The ability of the enterobacterial KatG enzyme to protect DHR from oxidation by peroxynitrite was investigated next. Recent literature suggests that peroxynitrite *per se* is not responsible for DHR oxidation; rather, it is the peroxynitrite-derived radicals, nitrogen dioxide and hydroxyl radicals (formed at 0.9 s^{-1}) that interact with DHR ([17] and references therein). Given the second-order rate constant and the concentrations of enzyme used in this assay (5–40 μM), KatG would be expected to intercept peroxynitrite partially. DHR and peroxynitrite were mixed with varying concentrations of KatG in order to determine experimentally whether the enzyme is able to protect DHR from oxidation. This assay was undertaken using peroxynitrite from Calbiochem® and also using peroxynitrite tetramethylammonium. When using the Calbiochem® peroxynitrite, which is likely to contain H_2O_2 as unused substrate, KatG seemed to afford little protection from oxidation (~93% oxidation), with a small increase in oxidation at 5 μM KatG (closed circles, Fig. 3C). H_2O_2 is unable to oxidise DHR alone however; in the presence of a catalyst, oxidation can occur [18,19]. Given the slight increase in oxidation at low KatG concentrations it was necessary to repeat this assay using peroxynitrite tetramethylammonium, which contains no H_2O_2. We found that under these conditions KatG is able to intercept peroxynitrite and thus reduce oxidation of DHR to ~83% of that in the absence of enzyme (closed diamonds, Fig. 3C). This is in contrast to bovine

liver catalase, which was able to reduce oxidation by peroxynitrite to ~13% with 15 μM enzyme (open circles, Fig. 3C). The previously reported protection offered by AhpC from *S.* Typhimurium [20] and bovine heart catalase [13] is also greater than that of *S.* Typhimurium KatG.

4. Discussion

Characterisation of KatG from *S.* Typhimurium has yielded the first kinetic parameters for the enzyme from this organism, which are within the range for other KatG proteins [21].

In this study KatG was found to enhance the catabolism of peroxynitrite, which is in agreement with data previously published for the *M. tuberculosis* enzyme. The second-order rate constant for the enterobacterial enzyme was around $4 \times 10^4\ M^{-1}\ s^{-1}$, approximately 35-fold lower than those for AhpC from *S. typhimurium* and bovine heart catalase [13,20].

A reduction in the absorbance of the Soret band upon reaction with peroxynitrite was accompanied by a red shift of the peak. No isosbestic wavelength was found between the native and shifted peaks; this suggests that both compound I and compound II may be formed, as seen in the peroxynitrite study of horseradish peroxidase [22]. In order for the enzyme to function catalytically, it must be recycled to native enzyme; one possibility for this is reduction by contaminant H_2O_2 found in Calbiochem® preparations. However, the use of peroxynitrite tetramethylammonium had no affect on the rate of the reaction, which suggests that H_2O_2 does not reduce the enzyme. It has previously been shown that peroxynitrite is able to cause the one-electron reduction of compound I of myeloperoxidase to compound II [23]. Compound II may be reduced by nitrite, $^{\cdot}NO_2$ (formed by peroxynitrite decay) or peroxynitrite to native enzyme [14]. However, nitrite levels up to 100-fold excess over peroxynitrite and KatG showed no indication of affecting the rate of peroxynitrite decay, so it is unlikely that this is a route of compound II reduction. Another possibility is that the enzyme–substrate complex directly converts peroxynitrite to nitrate [24]. Clearly the mechanism of this KatG–peroxynitrite reaction warrants further study, which is beyond the scope of the current paper.

Our findings show that KatG from *S.* Typhimurium is able to enhance the breakdown of peroxynitrite and may play a wider role in the defence against oxidative stress than first thought.

Acknowledgments

This work was supported by the Biotechnology and Biological Sciences Research Council (UK). The authors would like to thank Professor D.J. Kelly for use of stopped flow apparatus.

References

[1] Ferrer-Sueta, G. and Radi, R. (2009) Chemical biology of peroxynitrite: kinetics, diffusion, and radicals. ACS Chem. Biol. 4, 161–177.
[2] Imlay, J.A. (2008) Cellular defenses against superoxide and hydrogen peroxide. Annu. Rev. Biochem. 77, 755–776.
[3] Martinez, M.C. and Andriantsitohaina, R. (2009) Reactive nitrogen species: molecular mechanisms and potential significance in health and disease. Antioxid. Redox Signal. 11, 669–702.
[4] Alvarez, M.N., Piacenza, L., Irigoin, F., Peluffo, G. and Radi, R. (2004) Macrophage-derived peroxynitrite diffusion and toxicity to *Trypanosoma cruzi*. Arch. Biochem. Biophys. 432, 222–232.
[5] Claiborne, A. and Fridovich, I. (1979) Purification of the ortho-dianisidine peroxidase from *Escherichia coli* B – physiochemical characterization and analysis of its dual catalytic and peroxidatic activities. J. Biol. Chem. 254, 4245–4252.
[6] Hebrard, M., Viala, J.P.M., Meresse, P., Barras, F. and Aussel, L. (2009) Redundant hydrogen peroxide scavengers contribute to *Salmonella* virulence and oxidative stress resistance. J. Bacteriol. 191, 4605–4614.
[7] Poole, R.K. (2005) Nitric oxide and nitrosative stress tolerance in bacteria. Biochem. Soc. Trans. 33, 176–180.
[8] Wengenack, N.L., Jensen, M.P., Rusnak, F. and Stern, M.K. (1999) *Mycobacterium tuberculosis* KatG is a peroxynitritase. Biochem. Biophys. Res. Commun. 256, 485–487.
[9] Beers, R. and Sizer, I. (1951) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 8, 133–140.
[10] Hildebrandt, A.G. and Roots, I. (1975) Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent formation and breakdown of hydrogen peroxide during mixed-function oxidation reactions in liver-microsomes. Arch. Biochem. Biophys. 171, 385–397.
[11] Marcinkeviciene, J.A., Magliozzo, R.S. and Blanchard, J.S. (1995) Purification and characterization of the *Mycobacterium smegmatis* catalase peroxidase involved in isoniazid activation. J. Biol. Chem. 270, 22290–22295.
[12] Wood, P.M. (1984) Bacterial proteins with co-binding B-type or C-type heme functions and absorption spectroscopy. Biochim. Biophys. Acta 768, 293–317.
[13] Sahoo, R., Bhattacharjee, A., Majumdar, U., Ray, S.S., Dutta, T. and Ghosh, S. (2009) A novel role of catalase in detoxification of peroxynitrite in *S. cerevisiae*. Biochem. Biophys. Res. Commun. 385, 507–511.
[14] Gebicka, L. and Didik, J. (2009) Catalytic scavenging of peroxynitrite by catalase. J. Inorg. Biochem. 103, 1375–1379.
[15] Saha, A., Goldstein, S., Cabelli, D. and Czapski, G. (1998) Determination of optimal conditions for synthesis of peroxynitrite by mixing acidified hydrogen peroxide with nitrite. Free Radic. Biol. Med. 24, 653–659.
[16] Bohle, D.S., Glassbrenner, P.A. and Hansert, B. (1996) Synthesis of pure tetramethylammonium peroxynitrite. Nitric Oxide Pt. B 269, 302–311.
[17] Wardman, P. (2007) Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. Free Radic. Biol. Med. 43, 995–1022.
[18] Crow, J.P. (1997) Dichlorodihydrofluorescein and dihydrorhodamine 123 are sensitive indicators of peroxynitrite *in vitro*: implications for intracellular measurement of reactive nitrogen and oxygen species. Nitric Oxide: Biol. Chem. 1, 145–157.
[19] Royall, J.A. and Ischiropoulos, H. (1993) Evaluation of 2′,7′-dichlorofluorescin and dihydrorhodamine 123 as fluorescent probes for intracellular H2O2 in cultured endothelial cells. Arch. Biochem. Biophys. 302, 348–355.
[20] Bryk, R., Griffin, P. and Nathan, C. (2000) Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature 407, 211–215.
[21] Singh, R., Wiseman, B., Deemagarn, T., Jha, V., Switala, J. and Loewen, P.C. (2008) Comparative study of catalase-peroxidases (KatGs). Arch. Biochem. Biophys. 471, 207–214.
[22] Floris, R., Piersma, S.R., Yang, G., Jones, P. and Wever, R. (1993) Interaction of myeloperoxidase with peroxynitrite – a comparison with lactoperoxidase, horseradish peroxidase and catalase. Eur. J. Biochem. 216, 881.
[23] Furtmuller, P.G., Jantschko, W., Zederbauer, M., Schwanninger, M., Jakopitsch, C., Herold, S., Koppenol, W.H. and Obinger, C. (2005) Peroxynitrite efficiently mediates the interconversion of redox intermediates of myeloperoxidase. Biochem. Biophys. Res. Commun. 337, 944–954.
[24] Herold, S. and Shivashankar, K. (2003) Metmyoglobin and methemoglobin catalyze the isomerization of peroxynitrite to nitrate. Biochemistry (Mosc) 42, 14036–14046.
[25] Trujillo, M., Budde, H., Pineyro, M.D., Stehr, M., Robello, C., Flohe, L. and Radi, R. (2004) *Trypanosoma brucei* and *Trypanosoma cruzi* tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. J. Biol. Chem. 279, 34175–34182.
[26] Dubuisson, M. et al. (2004) Human peroxiredoxin 5 is a peroxynitrite reductase. FEBS Lett. 571, 161–165.
[27] Ogusucu, R., Rettori, D., Munhoz, D.C., Netto, L.E.S. and Augusto, O. (2007) Reactions of yeast thioredoxin peroxidases I and II with hydrogen peroxide and peroxynitrite: rate constants by competitive kinetics. Free Radic. Biol. Med. 42, 326–334.
[28] Briviba, K., Kissner, R., Koppenol, W.H. and Sies, H. (1998) Kinetic study of the reaction of glutathione peroxidase with peroxynitrite. Chem. Res. Toxicol. 11, 1398–1401.